

CLAIMS FILED UNDER ART. 34 PCT

1. A chimeric inhibitor protein of a protease comprising
 - a) an inhibiting polypeptidic sequence and
 - 5 b) at least one polypeptidic sequence of a substrate-enzyme interaction site specific for said protease, wherein said protease is a kallikrein.
2. The chimeric inhibitor protein of a protease of claim 1, characterized in that the polypeptidic sequence of a substrate-enzyme interaction site is a substrate active site sequence,
10 fragments thereof, a molecular chimera thereof, a combination thereof and/or variants thereof.
3. The chimeric inhibitor protein of a protease of claim 2, characterized in that the substrate active site sequence is a Reactive Serpin Loop sequence, fragments thereof, a molecular chimera thereof, a combination thereof and/or variants thereof.
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4. The chimeric inhibitor protein of a protease of claim 3, characterized in that the Reactive Serpin Loop sequence is selected from the group comprising the SEQ ID No 16, 17, 18, 19, 20, 21, 22, fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.
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5. The chimeric inhibitor protein of a protease of claims 1 to 4, characterized in that the kallikrein is an hK2 or an hK3 kallikrein protein.
6. The chimeric inhibitor protein of a protease of claim 5, characterized in that the
25 kallikrein is an hK2 kallikrein protein.
7. The chimeric inhibitor protein of a protease of claims 1 to 6, characterized in that the inhibiting polypeptidic sequence is an inhibiting polypeptidic sequence of a serine or cysteine protease.
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8. The chimeric inhibitor protein of a protease of claim 7, characterized in that said inhibiting polypeptidic sequence is a serpin sequence, fragments thereof, a molecular chimera thereof, a combination thereof and/or variants thereof.

9. The chimeric inhibitor protein of a protease of claim 8, characterized in that the serpin sequence is selected from the group comprising the α -1 antichymotrypsin (ACT), protein C inhibitor (PCI), α -1 antiproteinase (AAT), human α -1 antitrypsin-related protein precursor (ATR), α -2-plasmin inhibitor (AAP), human anti-thrombin-III precursor (ATIII), protease inhibitor 10 (PI10), human collagen-binding protein 2 precursor (CBP2), protease inhibitor 7 (PI7), protease inhibitor leuserpin 2 (HLS2), human plasma protease C1 inhibitor (C1 INH), monocyte/neutrophil elastase inhibitor (M/NEI), plasminogen activator inhibitor-3 (PAI3), protease inhibitor 4 (PI4), protease inhibitor 5 (PI5), protease inhibitor 12 (PI12), human plasminogen activator inhibitor-1 precursor endothelial (PAI-1), human plasminogen activator inhibitor-2 placental (PAI2), human pigment epithelium-derived factor precursor (PEDF), protease inhibitor 6 (PI6), protease inhibitor 8 (PI8), protease inhibitor 9 (PI9), human squamous cell carcinoma antigen 1 (SCCA-1), human squamous cell carcinoma antigen 2 (SCCA-2), T4-binding globulin (TBG), Megsin, and protease inhibitor 14 (PI14), fragments thereof, molecular chimeras thereof, combinations thereof and/or variants thereof.

10. The chimeric inhibitor protein protease of any of the preceding claims, characterized in said chimeric inhibitor protein of a protease is selected from the group comprising MD820, MD 62, MD 61, MD 67 and MD CI.

11. The chimeric inhibitor protein of a protease of claim 10, characterized in said chimeric inhibitor protein of a protease is MD 62 or MD 67.

12. A purified and isolated DNA sequence encoding the chimeric inhibitor protein of a protease according to any of the preceding claims.

13. The purified and isolated DNA sequence of claim 12, characterized in that the sequence is selected from the group comprising SEQ ID N° 1, SEQ ID N° 3, SEQ ID N° 5, SEQ ID N° 7, SEQ ID N° 9, SEQ ID N° 11 and SEQ ID N° 13.

14. An expression vector characterized in that it comprises the purified and isolated DNA sequence of claims 12 to 13.

15. The expression vector of claim 14, characterized in that it further comprises a promoter operably linked to the purified and isolated DNA sequence.

5 16. A eukaryotic or prokaryotic host cell transfected with the expression vector of claims 14 or 15.

17. A pharmaceutical composition characterized in that it comprises a chimeric inhibitor protein of a protease of any of claims 1 to 11 as an active agent, and optionally in combination with one or more pharmaceutically acceptable carriers.

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18. A method of treating or preventing a proteolysis-associated disorder in a mammal comprising administering to said mammal the pharmaceutical composition of claim 17.

15 19. The method of claim 18, characterized in that the disorder is a disorder in which hK2 kallikrein activity is detrimental.

20. The method of claim 18 or 19, characterized in that the disorder is a cancer, an autoimmune disorder, an inflammatory disorder or an infectious disorder.

20 21. The method of claim 20, characterized in that the cancer is prostate cancer, breast cancer or a metastatic cancer.

22. The method of claim 20, characterized in that the inflammatory disorder is Benign Prostatic Hypertrophy.

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23. Use of the pharmaceutical composition of claim 17 for the preparation of a medicament for the treatment or prevention of a proteolysis-associated disorder in a mammal.

30 24. Use according to claim 23, characterized in that the disorder is a disorder in which hK2 kallikrein activity is detrimental.

25. Use according to claims 23 or 24, characterized in that the disorder is a cancer, an autoimmune disorder, an inflammatory disorder or an infectious disorder.

26. Use according to claim 25, characterized in that the cancer is prostate cancer, breast
5 cancer or a metastatic cancer.

27. Use according to claim 25, characterized in that the inflammatory disorder is Benign Prostatic Hypertrophy.

10 28. A method for producing the chimeric inhibitor protein of a protease of claims 1 to 11, comprising the steps of

- a) selecting a polynucleotidic sequence encoding a substrate-enzyme interaction site specific for a protease,
- b) introducing said polynucleotidic sequence into a sequence encoding an inhibitor
15 protein of a serine or cysteine protease, so as to obtain a chimeric sequence,
- c) allowing expression of said chimeric sequence in a cell expression system under suitable conditions,
- d) and recovering the chimeric inhibitor protein of a protease.

20 29. The method of claim 28, characterized in that step a) is performed by phage-displayed library screening.

30. The method of claims 28 and 29, characterized in that the suitable conditions consist in culturing the cell expression system at a temperature between 10-40°C during 10-30 hours.

25 31. The method of claim 30, characterized in that the suitable conditions consist in a temperature of 16°C during 16 hours.

30 32. The method of claims 28 to 31, characterized in that step b) is achieved by separation after extraction of said chimeric inhibitor protein of a protease from the cell expression system.

33. The method of claim 32, characterized in that the separation of said chimeric inhibitor protein of a protease is achieved by affinity chromatography.

5 34. The method of claims 28 to 33, characterized in that the chimeric inhibitor protein of a protease is further assayed for its ability to inhibit the activity of the protease.

35. The method of claims 28 to 34, characterized in that the cell expression system is a eucaryotic or a prokaryotic cell.

10 36. The method of claim 35, characterized in that the prokaryotic cell is a bacterial cell.

37. A diagnostic kit for the detection of a protease in a specimen characterized in that it comprises any suitable purified and isolated DNA sequence selected from the group comprising SEQ ID N° 1, 3, 5, 7, 9, 11, 13, a sequence complementary thereof, fragments thereof, and/or variants thereof.

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38. A diagnostic kit for the detection of a protease in a specimen characterized in that it comprises a chimeric inhibitor of a protease according to claims 1 to 11.

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ABSTRACT

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The present invention relates to a chimeric inhibitor protein of a protease comprising an inhibiting polypeptidic sequence and at least one polypeptidic sequence of a substrate-enzyme interaction site specific for a protease.

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Other objects of the invention are to provide a purified and isolated DNA sequence encoding the chimeric inhibitor protein of a protease, an expression vector characterized in that it comprises said purified and isolated DNA sequence, a eukaryotic or prokaryotic host cell transformed with this expression vector and a method of producing a chimeric inhibitor protein.

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